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Process,specimen and device for making an analyte available for an investigation

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(58) Field of search

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Fig. 1

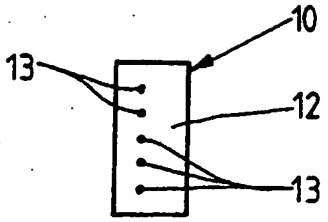


Fig. 2

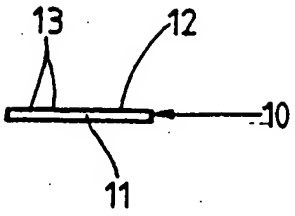


Fig. 3

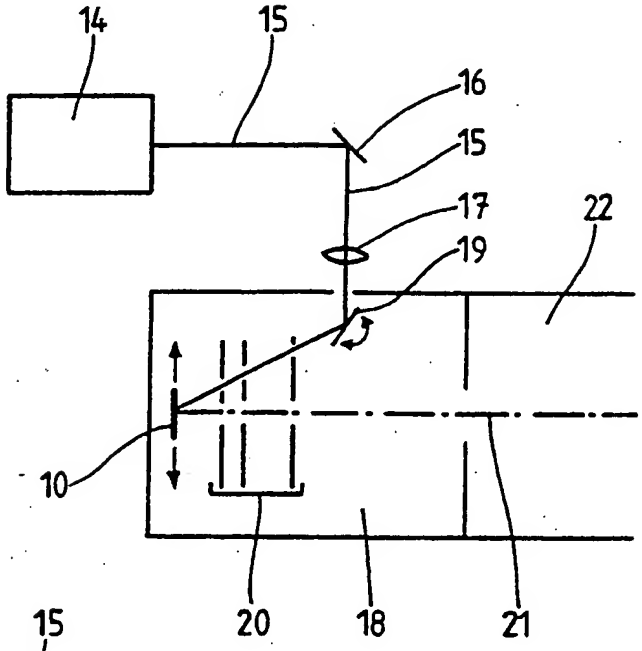
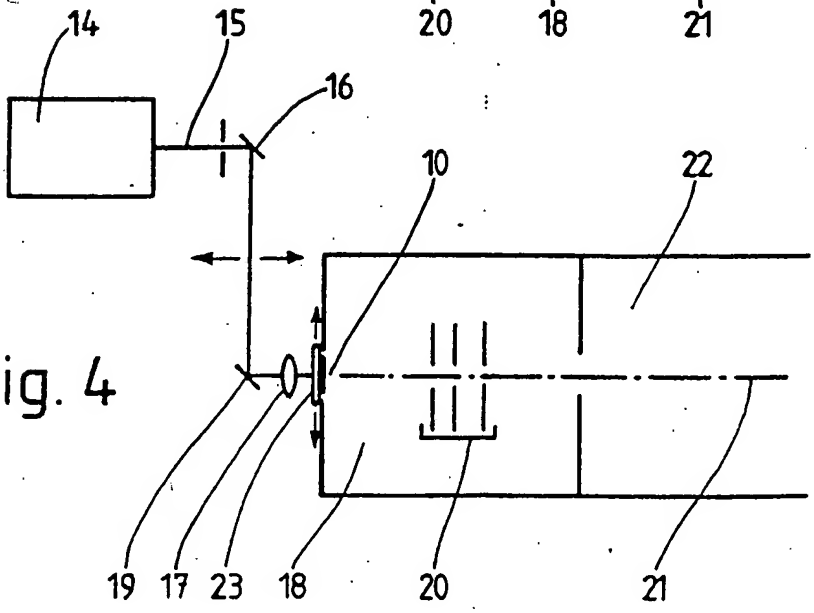


Fig. 4



PROCESS, SPECIMEN AND DEVICE FOR MAKING AN ANALYTE AVAILABLE FOR AN INVESTIGATION

The invention relates to a process for making an analyte available for an investigation, in which the analyte molecules are bound in an essentially two-dimensional layer on a surface of a specimen.

The invention furthermore relates to a specimen for use in the abovementioned process and a device for carrying out the process.

In many practical problems which are posed, an analyte which is to be (further) investigated is essentially in the form of a two-dimensional monolayer, and generally also immobilized, on a surface, or it is applied in this form to a surface which is suitable or to be made suitable. Examples of this are one- or two-dimensional electrophoresis gel plates, chromatography plates or sensor surfaces of biosensors.

The problem which arises for an investigation or further investigation, for example after chromatography or electrophoresis, of the analyte is that the analyte molecules must be specifically removed from the surface, for example for an investigation by mass spectrometry. In general terms, it is necessary to make the analyte molcules available for an investigation or further investigation.

Hence the object of the invention is to improve the process mentioned in the introduction for making an analyte available for an investigation.

According to a first aspect of the present invention there is provided a process for making an analyte available for an investigation comprising binding analyte molecules substantially in a monolayer on a surface of a substrate thereby forming a specimen which also includes a matrix for absorbing laser energy and for ionisation of the analyte molecules, and desorbing analyte molecule ions from the surface by laser desorption.

The process according to the invention can be used to remove analyte molecules in an advantageous manner specifically from a surface and to make them available for an investigation, for example by mass spectrometry.

Laser desorption, especially for preparation for mass

spectrometry is known per se for three-dimensional samples in which the analyte molecules are present in a volume distribution in a suitable matrix.

Laser desorption is now advantageously used according to the invention also for making an analyte available from a surface for an investigation. It is surprising that laser desorption can also be used for essentially two-dimensional surface samples.

The laser desorption according to the invention is made possible by use of a specimen which consists of the analyte and components for absorbing the laser energy. An example of a suitable absorbing component is nicotinic acid. However, other suitable examples are thymine, pyrazinecarboxylic acid, thiourea or vanillic acid.

The substance which is chosen as substrate on whose surface the analyte is bound can be one which itself is suitable for absorbing laser light, for example polycarbonate.

An essentially two-dimensional sample can be transferred (blotted) onto another surface for the (further) investigation, in which case the assignment of individual analyte zones is retained on the second surface. An example of a suitable substrate for surface blotting is nitrocellulose.

The analyte molecules can be bound via spacer molecules on the specimen surface, in which case the spacer molecules themselves can be absorbers of laser light, for example L-3,5-dinitrobenzoylphenylglycine, or it is possible to use spacer molecules which do not absorb laser light, for example propylamine.

The analyte molcules can be subjected to chemical reactions by means of chemical reagents before the laser desorption. For example, typical reagents for breaking down proteins are proteases, for example trypsin.

The substrate of the specimen can be chosen such that a chromatographical or electrophoretic separation of analytes in an analyte mixture can be carried out. A suitable separating substrate would be the polyacrylamide which is normally used in electrophoresis.

If separate zones of analyte molecules are present on a surface, it is possible to apply laser desorption successively to these zones by scanning laser light over these zones in succession. It is possible for this purpose to move a laser and the specimen relative to one

another.

According to a second aspect of the present invention there is provided a device for performing a process according to the first aspect of the present invention upon a specimen, comprising a laser for carrying out a laser desorption, the laser and the specimen being movable relative to one another whereby the specimen surface can be scanned with the laser beam.

Preferably the laser beam is arranged to be movable in a defined manner.

Preferably the specimen is arranged to be movable in a defined manner.

One or more guiding or deflecting elements may be provided for guiding or deflecting the laser beam, being arranged to be movable in such a way that the laser beam can be swept over analyte zones of the specimen.

Preferably a time and location control is provided for the laser scanning.

According to a third aspect of the present invention, there is provided a specimen for use in a process according to the first aspect of the present invention comprising analyte molecules bound substantially in a monolayer on a surface of a substrate, the specimen including a matrix which is suitable for the absorption of laser energy and for the ionisation of the analyte molecules.

Preferably zones of analyte molecules have been transferred (blotted) onto the substrate surface from another sample surface.

Preferably the substrate onto whose surface the analyte zones are transferred (blotted) is nitrocellulose.

The specimen may further comprise spacer molecules which bind the analyte molecules on the specimen surface.

Preferably the substrate is suitable for carrying out a chromatographic or electrophoretic separation of a mixture of various analytes.

The substrate may be, for example, polyacrylamide.

Exemplary embodiments from which further inventive features emerge are depicted in the drawings. The diagrams show in:

Fig. 1 a plan view of a specimen with which analyte molecules are made available for an investigation,

Fig. 2 a side view of the specimen shown in Fig. 1,

Fig. 3 a plan view of a device for carrying out a laser desorption for making analyte molecules available for an investigation according to a first exemplary embodiment of the invention and

Fig. 4 a plan view of a device for carrying out a laser desorption for making analyte molecules available for an investigation according to a second exemplary embodiment of the invention.

Fig. 1 shows a plan view of a specimen 10 which consists of a backing plate 11 (Fig. 2) and of a substrate layer 12 applied to the backing plate 11. Individual analyte zones 13 are indicated on the surface of

the substrate layer 12. In these analyte zones 13, analyte molecules are bound essentially two-dimensionally on the surface of the substrate 12. The different analyte zones 13 indicated in Fig. 1 might, for example, be derived from a single analyte zone of a mixture of analytes from which the analytes have been separated by chromatography. In this chromatography the distances migrated by the analytes in the individual analyte zones 13 on the substrate surface differ.

Apart from the analytes, the substrate layer 12 also has components which can absorb laser light. Components of this type can also be the substrate components themselves. It is possible in this way for the analytes in the specimen shown in Fig. 1 to be made available by laser desorption for an investigation or further investigation.

The specimen 10 shown in Fig. 1 can be an original sample, but it can also be a blot of an original sample, the analyte zones 13 having been transferred, while retaining their mutual correlation, from a surface onto the surface of the substrate layer 12 of the specimen 10.

Fig. 2 shows a side view of the specimen 10 shown in Fig. 1, from which it can be seen that the analyte zones 13 are present in an essentially two-dimensional layer.

Fig. 3 shows a plan view of an exemplary embodiment for a device with which analyte molecules in a specimen 10 can be made available by means of laser desorption for an investigation.

The device shown in Fig. 3 comprises a laser 14 which emits a laser beam 15. The laser beam 15 is deflected by means of a first reflecting mirror 16 and focused by means of a focusing lens 17. After the focusing, the laser beam 15 enters the vacuum chamber 18 of an ion source. A specimen 10, which can be, for example, attached to a specimen support, is located in this vacuum chamber 18 of the ion source. The laser beam 15 entering the vacuum chamber 18 is deflected a second time by a

second reflecting mirror 19. After this, the laser beam 15 impinges on the specimen 10.

In order for it to be possible to scan the individual analyte zones 13 of the specimen 10 with the laser beam 15, the specimen 10 is arranged to be displaceable in its specimen plane and/or the second deflecting mirror 19 is rotatably mounted, as is indicated in each case by arrows in Fig. 3.

An ion-optical system 20 of the ion source is located in the vacuum chamber 18 of the ion source and is used to extract, and focus to an ion beam 21, the analyte molecules or analyte ions which are removed by laser desorption from the specimen 10. The ion beam 21 emerges from the vacuum chamber 18 of the ion source into a vacuum chamber 22 of an analyzer with which the analyte molecules are investigated. The analyzer can be, for example, a mass spectrometer.

The device shown in Fig. 4 comprises a laser 14 which emits a laser beam 15. The laser beam 15 is directed by means of two reflecting mirrors 16 and 19 onto a transparent sample support 23, and focused via the focusing lens 17 into the plane of the specimen 10. The sample support 23 is transparent for the laser wavelength used and simultaneously serves for vacuum sealing of the vacuum chamber 18. The specimen 10 is located, as depicted in Fig. 2, on the side facing the analyzer.

The laser beam 15 can be scanned with the aid of the reflecting mirrors 16 and 19 over the analyte zone, and where the extent of the sample is relatively large it is possible to displace the sample support relative to the laser beam, as is indicated by corresponding arrows in Fig. 4.

An ion-optical system 20 of the ion source is again located in the vacuum chamber 18 of the ion source and is used to extract, and to focus to an ion beam 21, the analyte molecules or analyte ions which are removed by laser desorption from the specimen 10. The ion beam 21 emerges from the vacuum chamber 18 of the ion source into a vacuum chamber 22 of an analyzer with which the analyte

molecules are investigated. The analyzer can be, for example, a mass spectrometer.

Claims:

1. A process for making an analyte available for an investigation comprising binding analyte molecules substantially in a monolayer on a surface of a substrate thereby forming a specimen which also includes a matrix for absorbing laser energy and for ionisation of the analyte molecules, and disorbing analyte molecule ions from the surface by laser desorption.

2. A process according to Claim 1, wherein nicotinic acid is used as the matrix.

3. A process according to Claim 1 or Claim 2, wherein the substrate is itself suitable for absorbing laser light.

4. A process according to Claim 3, wherein the substrate is polycarbonate.

5. A process according to Claim 1 or Claim 2, wherein the matrix is applied to the substrate before the analyte and the analyte is applied on top of the matrix.

6. A process according to Claim 1 or Claim 2, wherein the matrix is applied to the substrate after and on top of the analyte.

7. A process according to Claim 5 or Claim 6, wherein the matrix is applied by spraying, centrifugation or vacuum deposition.

8. A process according to any preceding claim, wherein analyte zones are transferred (blotted), while retaining their correlation to one another, from another support down onto the surface.

9. A process according to Claim 8, wherein nitrocellulose is used as the substrate onto which transfer (blotting) is carried out.

10. A process according to any preceding claim, wherein the analyte

molecules are bound via spacer molecules on the substrate surface.

11. A process according to Claim 10, wherein propylamine is used as a spacer.

12. A process according to Claim 10, wherein the spacer molecules used are suitable for absorbing laser light.

13. A process according to Claim 12, wherein L-3,5-dinitrobenzoylphenylglycine is used as a spacer.

14. A process according to any preceding claim, wherein the analyte molecules are subjected to chemical reactions by means of chemical reagents before the laser desorption.

15. A process according to Claim 14, wherein a protease is used as a chemical reagent for breaking down proteins.

16. A process according to any preceding claim, wherein the binding of the analyte molecules to the substrate is loosened or destroyed shortly before the laser desorption.

17. A process according to any preceding claim, wherein the substance chosen as substrate is one which is suitable for carrying out a chromatographical or electrophoretic separation of a mixture of various analytes, and a separation of this type is carried out before the laser desorption.

18. A process according to Claim 17, wherein polyacrylamide is used as substrate for carrying out an electrophoresis.

19. A process according to Claim 17 or Claim 18, wherein zones of different analyte molecules which have been separated from one another by chromatography or electrophoresis are successively scanned and irradiated with laser light using a laser.

20. A process according to Claim 19, wherein the specimen and the

laser beam are moved relative to one another for the scanning.

21. A specimen for use in a process according to any preceding claim, comprising analyte molecules bound substantially in a monolayer on a surface of a substrate, the specimen including a matrix which is suitable for the absorption of laser energy and for the ionisation of the analyte molecules.

22. A specimen according to Claim 21, wherein zones of analyte molecules have been transferred (blotted) onto the substrate surface from another sample surface.

23. A specimen according to Claim 22, wherein the substrate onto whose surface the analyte zones are transferred (blotted) is nitrocellulose.

24. A specimen according to any one of Claims 21 to 23, further comprising spacer molecules which bind the analyte molecules on the specimen surface.

25. A specimen according to any one of Claims 21 to 24, wherein the substrate is suitable for carrying out a chromatographic or electrophoretic separation of a mixture of various analytes.

26. A specimen according to Claim 25, wherein the substrate is polyacrylamide.

27. A device when used in performing a process according to any one of Claims 1 to 20 upon a specimen, comprising a laser for carrying out a laser desorption, the laser and the specimen being movable relative to one another whereby the specimen surface can be scanned with the laser beam.

28. A device according to Claim 27, wherein the laser beam is arranged to be movable in a defined manner.

29. A device according to Claim 27 or 28, wherein the specimen is

arranged to be movable in a defined manner.

30. A device according to any one of Claims 27 to 29, wherein at least one guiding or deflecting element is provided for guiding or deflecting the laser beam and is arranged to be movable in such a way that the laser beam can be swept over analyte zones of the specimen.

31. A device according to any one of Claims 27 to 30, wherein a time and location control is provided for the laser scanning.

32. A device when used in performing the process of any one of claims 1 to 20 upon a specimen, substantially as hereinbefore described with reference to figures 3 and 4 of the accompanying drawings.

33. A specimen for use in a process for making an analyte available for an investigation, substantially as hereinbefore described with reference to figures 1 and 2 of the accompanying drawings.

34. A process for making an analyte available for an investigation, substantially as hereinbefore described with reference to the accompanying drawings.